The Veterinary Journal xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases

S.L. Benali [a], G.E. Lees [b], M.B. Nabity [c], R. Mantovani [d], F. Bonsembiante [a], L. Aresu [a,*]

[a] Department Comparative Biomedicine and Food Science, University of Padova, AGRIPOLIS – Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA
[c] Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA
[d] Department of Agronomy Food Natural Resources Animals & Environment, University of Padova, AGRIPOLIS – Viale dell'Università 16, 35020 Legnaro, PD, Italy

ARTICLE INFO

Article history:
Accepted 10 June 2013
Available online xxxx

Keywords:
Dog
Epithelial–mesenchymal transition
Fibrosis
Glomerulonephritis
Renal biopsy

ABSTRACT

Tubulointerstitial fibrosis (TIF) plays a central role in the progression to end-stage renal disease. Tubular epithelial cells (TECs) undergo epithelial–mesenchymal transition (EMT) and may contribute to the progression of TIF. Using immunohistochemistry, the primary aim of this study was to assess the expression of β-catenin, human leukocyte antigen-DR (HLA-DR) and vimentin in renal biopsies from dogs with spontaneous kidney diseases of varying severities. Morphological diagnosis, severity of inflammation, TIF, HLA-DR expression and clinicopathological variables were compared in dogs with renal injury to identify any potential relationship between the different factors; β-catenin down-regulation was used as a marker of EMT.

Fibrosis, HLA-DR expression, serum creatinine concentration (SCr), and urine protein-to-creatinine ratio (UPC) were all increased and β-catenin expression decreased in dogs with primary glomerular disease compared with dogs with acute tubular necrosis. HLA-DR expression by TECs was positively correlated to fibrosis, inflammation, UPC, and SCr. β-catenin expression was negatively correlated to fibrosis, inflammation and HLA-DR expression. The progression of renal failure correlated closely with tubulointerstitial damage. De novo HLA-DR expression associated with β-catenin down-regulation by TECs may represent a possible step in the progression of TIF and EMT.

Introduction

Kidney lesions can primarily involve the glomeruli, tubulointerstitium, or renal vessels. However, regardless of the initiating site of injury, all compartments often eventually become affected (Finco et al., 1999). Serum creatinine concentration (SCr), urinalysis findings, and renal biopsy are diagnostic tools commonly used to evaluate renal damage, guide treatment decisions, and determine prognosis (Lees, 2004).

Tubulointerstitial damage (TID), independent of the initial cause of the injury, plays a central role in the progression of renal disease, leading to an irreversible decline in renal function and ultimately resulting in end-stage renal disease (ESRD) (MacDougall et al., 1986). TID is characterized by loss of renal tubules, increased number of interstitial myofibroblasts, and interstitial accumulation of extracellular matrix (ECM) usually with inflammatory cell infiltrates (Zeisberg and Duffield, 2010). Various studies have demonstrated a potential role of epithelial-to-mesenchymal transition (EMT) in the development of renal interstitial fibrosis both in humans and dogs (Rastaldi et al., 2002; Aresu et al., 2007). EMT is observed in three distinct biological settings with very different functional consequences. In type 1 EMT, cells lose their epithelial phenotype, acquire mesenchymal features, and migrate to generate new organs in the embryo. This mechanism is replicated in Type 3 EMT, where cancerous cells acquire the ability to disseminate by metastasis. In type 2 EMT, epithelial cells subject to injury represent a source of new fibroblasts in the interstitium.

Type 2 EMT in the kidney is described as an organ repair-associated event (Kalluri and Weinberg, 2009). One of the first events leading to EMT is down-regulation of adhesion molecules (E-cadherin and β-catenin) that normally mediate cell–cell and cell-basement membrane interactions and attachment of epithelial cells (Tian et al., 2011). This is followed by detachment of cells, acquisition of cell mobility, and expression of de novo mesenchymal markers (Rastaldi et al., 2002; Kalluri and Neilson, 2003). Direct evidence for the active role of β-catenin as an integral component of intercellular junctions has been demonstrated, and the loss of its

* Corresponding author. Tel.: +39 049 827 2963.
E-mail address: luca.aresu@unipd.it (L. Aresu).

1090-0233/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2013.06.006

cytoplasmatic expression is considered a useful marker of EMT (Aresu et al., 2008b; Tian et al., 2011).

Class II major histocompatibility complex (MHC) molecules are heterodimeric transmembrane proteins that are responsible for activation of antigen-specific T helper lymphocytes during antigen processing by various antigen-presenting cells (APCs) (Frei et al., 2010). MHC is also known as human leukocyte antigen (HLA). Human class II genes are encoded by genes in the HLA-D region, which includes three subregions called DQ, DP, and DR. The HLA-DR subregions are highly conserved in nearly all mammals, including the dog (Yuhki et al., 2007). In humans, tubular epithelial cells (TECs) are able to express class II MHC under certain conditions, such as in renal allografts during graft-versus-host reactions (Cheng et al., 1989). Class II MHC expression in TECs has also been described in a mouse model of systemic lupus erythematosus and in canine chronic interstitial nephritis (Wuthrich et al., 1989; Vilafranca et al., 1995).

The aim of the present study was to assess the relationship between β-catenin and HLA-DR expression by TECs as assessed by immunohistochemistry (IHC) and the severity of chronic tubulointerstitial damage (CTID) in dogs with naturally-occurring renal disease due to various causes.

Materials and methods

Case selection and clinical chemistry

Canine renal biopsies ($n = 28$) were selected from routine diagnostic cases submitted to the Histopathology Service, Department of Comparative Biomedicine and Food Science, University of Padova between January 2009 and December 2011. All biopsies had a minimum of 12 evaluable glomeruli in 10 serial histological sections (3 μm in thickness) and were composed of only cortex, as the medulla is usually avoided during biopsy procedures to reduce the risk of haemorrhage. Five renal biopsies from dogs with no signs or history of renal disease were used as controls. SCr and urine protein–creatinine ratio (UPC) were provided by the referring clinicians at the time of renal biopsy submission. Proteinuria was defined as UPC > 0.5 and azotaemia by SCr > 123.8 μmol/L.

Classification of renal biopsies

Renal biopsies were evaluated by light microscopy, immunofluorescence, and electron microscopy as previously described (Aresu et al., 2008a; Aresu et al., 2012). Based on biopsy findings, each case was assigned to one of five diagnostic categories as follows: (1) acute tubular necrosis (ATN), (2) amyloidosis (AMYL), (3) chronic glomerulosclerosis (CGS), (4) membranoproliferative glomerulonephritis (MPGN), and (5) mesangioproliferative glomerulonephritis (MeGN).

ATN was characterized by primary damage to proximal tubules, which showed degeneration and necrosis of epithelial cells. Multifocally, the injured tubules contained sloughed tubular epithelial cytoplasm, pyknotic nuclear debris and mineralization. AMYL was characterized by glomeruli with diffuse and global mesangial expansion by an amorphous congophilic material consistent with amyloid. CGS was characterized by glomeruli with moderate to severe global mesangial matrix increase, with or without hypercellularity, and absence of immune deposits. MPGN was characterized by glomeruli that exhibited increased mesangial matrix and cellularity, thickening of glomerular capillary walls with endothelial hypertrophy, and immune deposits in the capillary wall and mesangium. MeGN was characterized by glomeruli with mesangial hypercellularity and increased mesangial matrix with no involvement of the capillary lumen, together with immune deposits in the mesangium as demonstrated on ultrastructural examination.

Histological indices of interstitial inflammation and fibrosis

Interstitial inflammation was evaluated using haematoxylin and eosin (HE)-stained sections. The inflammation index was determined by counting the number of inflammatory cell nuclei (lymphocytes, plasma cells and neutrophils) in 10 randomly selected microscopic fields of renal cortex with a 20× objective. The index for each case was expressed as the average number of inflammatory cells per field. Interstitial fibrosis was evaluated using Masson's Trichrome-stained sections. Interstitial fibrosis was evaluated in the cortex and severity of cortical interstitial fibrosis (CIF) was graded by examining 20 randomly selected fields with a 20× objective. A semi-quantitative assessment of the extent of fibrosis for each field was done as follows: grade 0: normal tubulointerstitium; grade 1: <25%; grade 2: 26–50%; grade 3: >51% (Aresu et al., 2007). The final score for each biopsy was the total of the scores for each field ranging from 0 to 60 (Nabity et al., 2012).

Immunohistochemical evaluation of tubular epithelial cell phenotypic markers

For IHC, serial paraffin sections (3 μm) were placed on surface-coated slides (Superfrost Plus). Slides were incubated at 37 °C for 30 min before the immunostaining procedures. Staining was performed using an automatic immunostainer (Ventana Benchmark XT, Roche-Diagnostics), which uses a kit with a secondary antibody with a horseradish peroxidase (HRP)-conjugated polymer that binds mouse and rabbit primary antibodies (ultraViews Universal DAB, Ventana Medical System). All reagents were dispensed automatically except for the primary antibody, which was dispensed by hand.

A mouse monoclonal antibody against the α-chain of human HLA-DR (Clone TAL.1B5, Dako, dilution 1:50) was used. The antibody was tested in canine lymph nodes, tonsils and thymus to determine cross-species reactivity. A single band of 34 kDa was identified by immunoblots using canine peripheral lymphocytes. Cross-reactivity with canine tissue was also confirmed by previous studies (Darbès et al., 1997; German et al., 1998). A mouse monoclonal antibody against human β-catenin (clone 14, Transduction Laboratories, dilution 1:100) was used to detect β-catenin expression and cross-reactivity of this antibody has previously been tested (Aresu et al., 2008b). Tissues were incubated with the primary antibody for 32 min at room temperature (RT) for HLA-DR and for 24 min at 42 °C for β-catenin. Slides were counterstained with Mayer's haematoxylin. Interstitial dendritic cells and epithelial cells in normal renal tissue were used as positive controls for HLA-DR and β-catenin, respectively. Negative controls were performed by replacing the primary antibody with antibody diluent.

Quantification of HLA-DR and β-catenin expression was assessed by counting the number of positively labelled and non-stained TECs in 10 randomly selected fields with a 40× objective. Expression of HLA-DR and β-catenin was determined by calculating the percentage of positively labelled cells among the total as follows:

$$\frac{\text{positively labelled cells}}{(\text{positively labelled cells} + \text{non-stained TECs})} \times 100$$

Double immunolabeling for HLA-DR and vimentin (V9 clone, monoclonal mouse, Dako) was performed on selected representative cases using an automatic immunostainer (Ventana Benchmark XT) according to the manufacturer's instructions. Tissues were incubated with HLA-DR as described above, followed by incubation with vimentin (diluted 1:150 and incubated 20 min at RT). Peroxidase activity was demonstrated using ultraViews universal diaminobenzidine (DAB, Ventana Medical System, brown to black chromogen reaction) for vimentin. For HLA-DR detection, ultraView universal alkaline phosphatase red detection kit was used (red chromogen reaction). Slides were counterstained with periodic acid Schiff (PAS) to highlight tubular basement membranes.

Statistical analysis

One-way analysis of variance (ANOVA) using the GLM procedure of the SAS Institute was carried out to assess relationships between renal disease diagnostic categories, magnitudes of azotaemia and proteinuria, and histologic indices of interstitial inflammation and fibrosis. Effects of different disease categories (ATN, AMYL, CGS, MPGN, MeGN, and NORMAL) on CIF grade, inflammation index, SCr, and UPC were examined. The degrees of freedom of the different disease categories were decomposed by a multiple comparisons test using the Bonferroni adjustment method (SAS Institute) to analyse the relative significance of each disease category within each analysed variable. Correlation and linear regression analyses were carried out with the CORR and REG procedures of SAS, respectively (SAS Institute), comparing HLA-DR with CIF grade, inflammation index, SCr, UPC, and β-catenin expression. Correlation and linear regression analysis were also carried out for CIF grade, inflammation score, SCr, and UPC in relation to β-catenin expression (i.e., using β-catenin expression as a dependent variable). Lastly, correlation and linear regression analysis were also conducted for inflammation score, SCr, and UPC in relation to CIF grade (i.e., using CIF grade as a dependent variable).

Results

Dogs, diagnostic categories, and clinical chemistry findings

A total of 33 canine renal biopsies were examined (28 affected dogs, 5 controls). The 28 dogs with renal disease were composed of 12 females, 15 males, and 1 unspecified. These dogs ranged in age from 1 to 13 years with a median of 6, and Boxer was the most represented breed (6/28 dogs). Primary glomerular lesions were diagnosed in 21 biopsies: 9 CGS, 7 MPGN, 3 MeGN and 2 AMYL. In seven dogs the histological diagnosis was primary ATN. The controls dogs were 4 month-old Beagles, 3 males and 2 females.

All but one of the affected dogs were azotemic (SCr > 123.8 μmol/L) and all but two were proteinuric (UPC > 0.5). Clinical data are shown in Table 1.

Please cite this article in press as: Benali, S.L., et al. De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases. The Veterinary Journal (2013), http://dx.doi.org/10.1016/j.tvjl.2013.06.006

Table 1
Comparison of clinical and histopathological variables according to diagnostic categories.

Diagnostic categories[a]	Normal	ATN	AMYL	MPGN	MeGN	CGS
Number of dogs	5	7	2	7	3	9
sCr[b]						
Mean	70.7	141.4	1034.3	689.5	848.6	512.7
Median	79.6	132.6	1034.3	769.1	1007.8	618.8
Range	61.9–97.2	97.2–185.6	1007.8–1069	300.6–919.4	468.5–742.6	132.6–742.6
UPC[c]						
Mean	0.24	1.45	3.6	3.6	4.8	2.5
Median	0.2	0.7	3.6	3.0	5.9	2.2
Range	0.2–0.3	0.3–1.2	3.1–4.1	1.8–6.5	1.9–6.7	1.0–4.1
Inflammation index[d]						
Mean	0	5.41	77.1	39.4	54.1	34.3
Median	0	5.1	77.1	35.5	65.4	33.1
Range	0	3.5–8.9	75.3–78.8	20.5–80.9	22.3–74.7	3.2–75.7
CIF score[e]						
Mean	5.6	11.6	50.5	32.3	40.0	31.2
Median	5.0	11.0	50.5	30.0	46.0	34.0
Range	12–53	8–55	8–15	22–52	51–52	5–7

[a] ATN, acute tubular necrosis; AMYL, amyloidosis; MPGN, membranoproliferative glomerulonephritis; MeGN, mesangioproliferative glomerulonephritis; CGS, chronic glomerulosclerosis.
[b] sCr: serum creatinine (μmol/L).
[c] UPC: urine protein–creatinine ratio.
[d] Inflammation index: average number of inflammatory cells per field in 10 fields (200×).
[e] CIF, chronic interstitial fibrosis.

Histological indices of interstitial inflammation and fibrosis

Inflammation was present in all dogs with renal disease. In dogs with acute tubular damage, the infiltrate was mild and multifocal, mainly composed of lymphocytes and rare neutrophils. In dogs with glomerular disease, the inflammation index was greater and mainly composed of lymphocytes and plasma cells (Table 1). In dogs with ATN, the amount of fibrosis was significantly lower compared with the other disease categories ($P < 0.01$). Independently of the histological diagnosis, the CIF grade was associated with the inflammation index ($r = 0.986$), UPC ($r = 0.914$), and sCr ($r = 0.904$) ($P < 0.05$).

Expression of HLA-DR and β-catenin in tubular epithelial cells

In control dogs, the expression of HLA-DR was restricted to the cytoplasm of dendritic cells within the renal interstitium. In all control dogs and one dog with minimal tubulointerstitial involvement, TECs were negative for HLA-DR staining (Fig. 1). HLA-DR labelling was present in TECs in all other dogs (Fig. 2). The intensity of immunostaining was moderate to strong and homogenous in the cytoplasm. Scattered lymphocytes and macrophages were also positive for HLA-DR. The number of HLA-DR positive TECs was significantly increased in biopsies with higher severity of CIF and

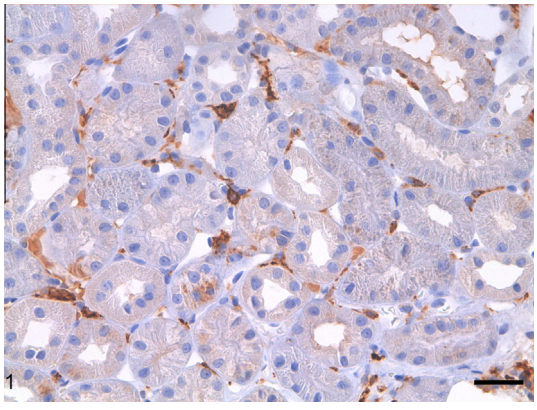

Fig. 1. Dog, renal biopsy, control. Diffuse cytoplasmatic HLA-DR expression is evident in interstitial dendritic cells. Immunohistochemical staining for HLA-DR, haematoxylin counterstain. (Bar, 50 μm).

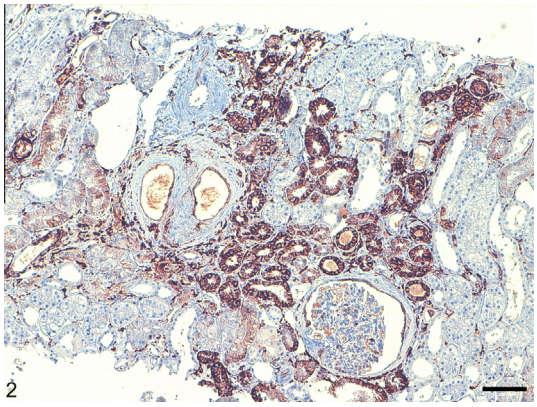

Fig. 2. Dog, renal biopsy, membranoproliferative glomerulonephritis. HLA-DR expression is detectable multifocally in the cytoplasm of proximal tubular epithelial cells. Immunohistochemical staining for HLA-DR, haematoxylin counterstain. (Bar, 100 μm).

Table 2
Comparison of immunohistochemical results according to diagnostic categories.

Diagnostic Category[a]	Normal	ATN	AMYL	MPGN	MeGN	CGS
Number of dogs	5	7	2	7	3	9
HLA-DR expression in TECs (% positively labelled cells)[b]						
Mean	0	4	64	41	5	33
Median	0	5	64	34	6	39
Min–Max	0	0–8	64–64	4–84	28–64	0–62
β-Catenin expression in TECs (% positively labelled cells)[b]						
Mean	97	90	30	59	48	62
Median	97	90	30	62	39	61
Min–Max	96–99	87–92	29–31	21–74	35–68	28–92

[a] ATN, acute tubular necrosis; AMYL, amyloidosis; MPGN, membranoproliferative glomerulonephritis; MeGN, mesangioproliferative glomerulonephritis; CGS, chronic glomerulosclerosis;
[b] TECs: tubular epithelial cells.

Please cite this article in press as: Benali, S.L., et al. De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases. The Veterinary Journal (2013), http://dx.doi.org/10.1016/j.tvjl.2013.06.006

higher inflammation index, and was highly correlated with UPC (Tables 1 and 2). Multifocally, TECs showed more intense immunoreactivity for HLA-DR when adjacent to inflammatory cells (Fig. 3).

Uniform membranous and cytoplasmatic β-catenin staining was observed in TECs in control dogs. A reduction of β-catenin expression was observed in the different renal diseases (Table 2). The number of β-catenin positive TECs was negatively correlated with the severity of inflammation ($r = -0.981$), CIF grade ($r = -0.984$), and with UPC ($r = -0.906$) and SCr ($r = -0.901$). Moreover, HLA-DR and β-catenin expression by TECs were negatively correlated (Figs. 4–7).

Evaluation of double immunostaining for HLA-DR and vimentin showed four different patterns: (1) tubules with a mixture of cells positive for either HLA-DR or vimentin, (2) tubules with only vimentin-positive cells, (3) tubules with only HLA-DR positive cells, and (4) tubules with co-localization of both markers in the same cell (Fig. 8). In control biopsies, TECs were not immunolabelled by either marker.

Discussion

The present study investigated the immunohistochemical expression of HLA-DR and β-catenin in renal biopsies from dogs with various kidney diseases. Association of expression of these markers with clinico-pathological data, as well as with measures of interstitial inflammation and fibrosis was examined. The inflammation index and CIF grade were significantly increased in dogs with primary glomerular diseases compared to dogs with primary acute tubular injury. No differences were evident for CIF grade and inflammation index among the CGS, MPGN, and MeGN disease categories.

Acute tubular damage is frequently characterized by degeneration and necrosis of tubules with no involvement of the interstitium, while glomerular diseases are associated with a variable increase in extracellular matrix, fibroblasts, and inflammatory cells in the interstitium at the time of biopsy. One possible explanation for the lack of tubulointerstitial changes in the ATN cases may be the rapid clinical onset of the disease in these dogs, since all ATN dogs were biopsied within a few days of the initial injury. With primary glomerular diseases, compensatory mechanisms such as glomerular hypertrophy and hyperfiltration are described to maintain kidney function for some time during the development of the primary process; therefore, secondary tubulointerstitial lesions may already be chronic and advanced by the time clinical signs become evident (Finco et al., 1999).

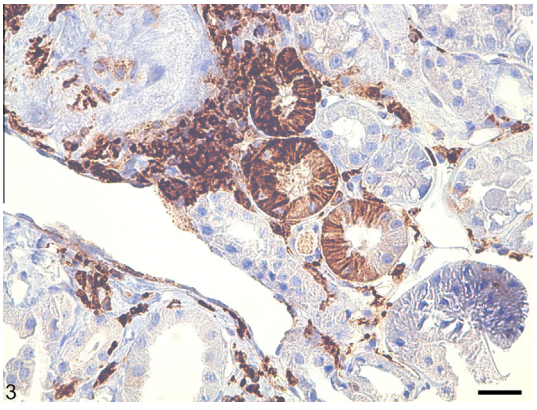

Fig. 3. Dog, renal biopsy, membranoproliferative glomerulonephritis. HLA-DR expression is diffusely detectable in lymphocytes and tubules adjacent to inflammation. Immunohistochemical staining for HLA-DR, haematoxylin counterstain. (400×; Bar, 50 μm).

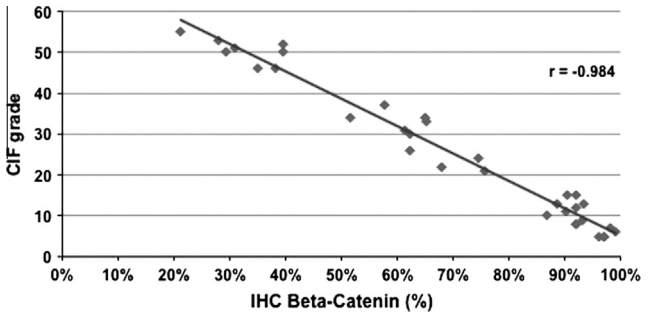

Fig. 4. Correlation between chronic interstitial fibrosis (CIF) grade and percentage of beta-catenin positive immunolabelled tubular epithelial cells (%).

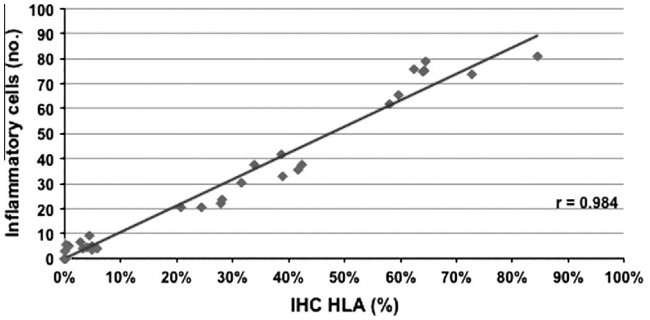

Fig. 5. Correlation between inflammation index and percentage of HLA-DR positive immunolabelled tubular epithelial cells (%).

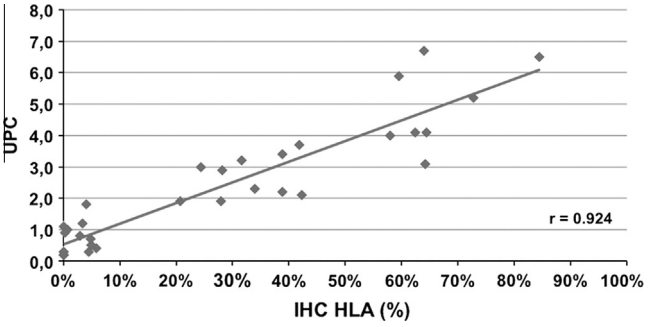

Fig. 6. Correlation between UPC and percentage of HLA-DR positive immunolabelled tubular epithelial cells (%).

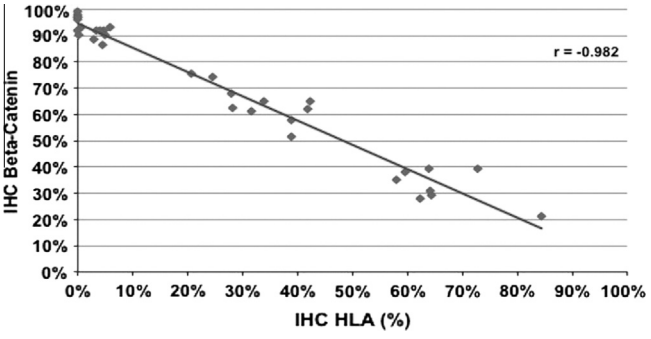

Fig. 7. Correlation between percentage of beta-catenin positively immunolabelled tubular epithelial cells (%) and percentage of HLA-DR positively immunolabelled tubular epithelial cells (%).

UPC and SCr were positively correlated with the severity of CIF and inflammation. However, these parameters were normal or

Please cite this article in press as: Benali, S.L., et al. De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases. The Veterinary Journal (2013), http://dx.doi.org/10.1016/j.tvjl.2013.06.006

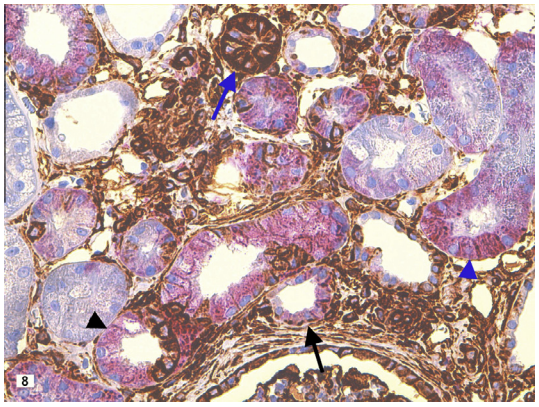

Fig. 8. Dog, renal biopsy, chronic glomerulosclerosis. Different pattern are present. (1) HLA-DR and vimentin expression in a single tubule (black arrow-head). (2) HLA-DR and vimentin co-localization in the cytoplasm of epithelial tubular cells (black arrow). (3) Diffuse vimentin expression in a tubule (blue arrow). (4) Diffuse HLA-DR expression in a tubule (blue arrow-head). Double immunostaining for HLA-DR (red signal) and vimentin (brown signal), periodic acid Schiff (PAS) counterstain (400×).

only slightly increased in dogs with mild CIF and inflammation, supporting their insensitivity for detecting early renal disease. This finding supports the need for additional more sensitive biomarkers of early damage in canine renal disease as pointed out in recent studies (Meyer et al., 2010; Smets et al., 2010; Slocum et al., 2012; Nabity et al., 2012).

The aim of this work was to confirm that decreased β-catenin expression is correlated with tubulointerstitial damage as has been previously described in canine EMT (Aresu et al., 2007) and to examine whether HLA-DR expression by TECs may be part of EMT. As expected, HLA-DR was expressed by interstitial dendritic cells, while TECs were negative in the control kidneys. Importantly, de novo expression of HLA-DR in the cytoplasm of TECs was found in the diseased kidneys and this expression was correlated with the CIF grade, UPC, and inflammation index. This result suggests a possible progressive transition of TECs to APCs. One possible explanation for this process is that some of the excess proteins (e.g. albumin) filtered by the glomerulus in primary glomerular diseases are physiologically reabsorbed by TECs. The proteins absorbed at the luminal membrane are endocytosed and concentrated within vesicles at the apical border of tubular cells. An overload of filtered protein results in production of chemokines, cytokines, and growth factors able to promote fibrosis and EMT (Abbate et al., 1998). Our results show a possible involvement of TECs in exposing the protein antigens with subsequently presentation of class II MHC, which may then exacerbate or perpetuate interstitial inflammation, particularly in glomerular diseases.

Interestingly, more intense HLA-DR immunostaining was found in TECs adjacent to inflammatory cells, most of which were lymphocytes. This may support the possibility that TECs may be able to directly interact with and activate T helper cells. Different cells (i.e. macrophages, B lymphocytes, and dendritic cells) are known to constitutively express HLA-DR (Kelley and Singer, 1993). Other cell types (i.e. intestinal epithelial cells, endothelial cells, chondrocytes, and thyroid epithelial cells) have been shown to function in a limited context as APCs (Wuthrich et al., 1989; German et al., 1998; Roda, 2010). Their efficiency is considered low and they are referred to as non-professional APCs. Based on the results of the present study, canine TECs could also be considered as non-professional APCs.

We found that β-catenin showed diffuse membranous and cytoplasmic expression in virtually of all TECs in control kidneys, and progressive loss of staining correlated with worsening of the CIF grade and higher inflammation index. β-Catenin plays an essential role in maintaining the structural integrity and polarity of epithelial cells, and its decreased expression may reduce adherens junction formation and lead to epithelial transdifferentiation. In fact, in the early phase of EMT, the expression of protein constituents of the tight junctions is decreased in epithelial cells. Subsequently, epithelial cells lose their intercellular contacts and polarity and undergo cytoskeleton reshaping by expressing proteins specific to mesenchymal cells, such as vimentin. In contrast to observations in human kidneys, the EMT of TECs in dogs seems to be characterized by de novo expression of vimentin rather than smooth muscle actin (Aresu et al., 2007).

The negative correlation between expression of HLA-DR and β-catenin by TECs observed in this study suggests the possibility of a previously unrecognized phase in the EMT process. To further investigate this observation, a double IHC protocol for vimentin and HLA-DR was used. The findings showed several different patterns of expression that may be consistent with the following three phase hypothesis: (1) when TECs express only HLA-DR, they act as APCs, presumably preceding the mesenchymal phase of EMT; (2) when TECs express both markers, a transition from one phase to another is occurring; (3) when TECs express only vimentin, the complete EMT process has occurred.

Conclusions

This study confirms the progressive decrease in β-catenin expression in TECs during tubulointerstitial damage associated with fibrosis and inflammation. De novo expression of HLA-DR in TECs could contribute to the recruitment of inflammatory cells and vimentin expression by TECs may be considered as a step during EMT. Further investigations on the role of HLA-DR may be helpful for determining novel therapeutic strategies to slow or arrest progression of renal diseases.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

Abbate, M., Zoia, C., Corna, D., Capitanio, M., Bertani, T., Remuzzi, G., 1998. In Progressive overload of tubular cells with filtered proteins translates glomerular permeability dysfunction into cellular signals of interstitial inflammation. Journal of the American Society of Nephrology 9, 1213–1224.

Aresu, L., Rastaldi, M.P., Scanziani, E., Baily, J., Radaelli, E., Pregel, P., Valenza, F., 2007. Epithelial–mesenchymal transition (EMT) of renal tubular cells in canine glomerulonephritis. Virchows Archive 451, 937–942.

Aresu, L., Pregel, P., Bollo, E., Palmerini, D., Sereno, A., Valenza, F., 2008a. Immunofluorescence staining for the detection of immunoglobulins and complement (C3) in dogs with renal disease. Veterinary Record 163, 679–682.

Aresu, L., Rastaldi, M., Pregel, P., Valenza, F., Radaelli, E., Scanziani, E., Castagnaro, M., 2008b. Dog as model for down-expression of E-cadherin and b-catenin in tubular epithelial cells in renal fibrosis. Virchows Archive 453, 617–625.

Aresu, L., Benali, S., Ferro, S., Vittone, V., Gallo, E., Brovida, C., Castagnaro, M., 2012. Light and electron microscopic analysis of consecutive renal biopsy specimens from Leishmania-seropositive dogs. Veterinary Pathology. http://dx.doi.org/10.1177/0300985812459336.

Cheng, H., Nolasco, F., Cameron, J.S., Hildreth, G., Neild, G., Hartley, B., 1989. Nephrology Dialysis transplantation original article of and phenotype HLA-DR display by renal tubular epithelium infiltrate in interstitial nephritis. Nephrology Dialysis Transplantation 4, 205–215.

Darbès, J., Majzoub, M., Hermanns, W., 1997. Evaluation of the cross-reactivity between human and feline or canine leucocyte antigens using commercially available antibodies. Journal of Veterinary Diagnostic Investigation 9, 94–97.

Finco, D.R., Brown, S., Brown, C., Crowell, W., Cooper, T., Barsanti, J., 1999. Progression of chronic renal disease in the dog. Journal of Veterinary Internal Medicine 13, 516–528.

Frei, R., Steinle, J., Birchler, T., Loeliger, S., Roduit, C., Steinhoff, D., Seibl, R., Büchner, K., Seger, R., Reith, W., et al., 2010. MHC class II molecules enhance toll-like receptor mediated innate immune responses. Cytokine 5, 1–6.

Please cite this article in press as: Benali, S.L., et al. De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases. The Veterinary Journal (2013), http://dx.doi.org/10.1016/j.tvjl.2013.06.006

German, J., Bland, P.W., Hall, E.J., Day, M.J., 1998. Expression of major histocompatibility complex class II antigens in the canine intestine. Veterinary Immunology and Immunopathology 61, 171–180.

Kalluri, R., Neilson, E.G., 2003. Epithelial–mesenchymal transition and its implications for fibrosis. Journal of Clinical Investigation 112, 1776–1784.

Kalluri, R., Weinberg, R.A., 2009. The basics of epithelial–mesenchymal transition. Journal of Clinical Investigation 119, 1420–1428.

Kelley, V.R., Singer, G.G., 1993. The antigen presentation function of renal tubular epithelial cells. Experimental Nephrology 1, 102–111.

Lees, G.E., 2004. Early diagnosis of renal disease and renal failure. Veterinary Clinics of North America: Small Animal Practice 34, 867–885.

MacDougall, D.F., Cook, T., Steward, A.P., Cattell, V., 1986. Canine chronic renal disease: prevalence and types of glomerulonephritis in the dog. Kidney International 29, 1144–1151.

Meyer, E., Duchateau, L., Daminet, S., 2010. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. Journal of Veterinary Internal Medicine 24, 65–72.

Nabity, M., Lees, G., Cianciolo, R., 2012. Urinary biomarkers of renal disease in dogs with X-linked hereditary nephropathy. Journal of Veterinary Internal Medicine 26, 282–293.

Rastaldi, M., Ferrario, F., Giardino, L., 2002. Epithelial–mesenchymal transition of tubular epithelial cells in human renal biopsies. Kidney International 62, 137–146.

Roda, G., 2010. Intestinal epithelial cells in inflammatory bowel diseases. World Journal of Gastroenterology 16, 4264.

Slocum, J.L., Heung, M., Pennathur, S., 2012. Marking renal injury: Can we move beyond serum creatinine? Translational Research 159, 277–289.

Smets, P., Meyer, E., Maddens, B.E.J., Duchateau, L., Daminet, S., 2010. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. Journal of Veterinary Internal Medicine 24, 65–72.

Tian, X., Liu, Z., Niu, B., Zhang, J., Tan, T.K., Lee, S.R., Zhao, Y., Harris, D.C.H., Zheng, G., 2011. E-cadherin/β-catenin complex and the epithelial barrier. Journal of Biomedicine and Biotechnology. http://dx.doi.org/10.1155/2011/567305.

Vilafranca, M., Wohlsein, P., Trautwein, G., 1995. Expression of class II major histocompatibility complex molecules in renal tubular epithelial cells of canine kidneys affected with tubulointerstitial nephritis. Research in Veterinary Science 59, 114–117.

Wuthrich, R., Yui, M., Mazoujian, G., 1989. Enhanced MHC class II expression in renal proximal tubules precedes loss of renal function in MRL/lpr mice with lupus nephritis. American Journal of Pathology 134, 45–51.

Yuhki, N., Beck, T., Stephens, R., Neelam, B., O'Brien, S.J., 2007. Comparative genomic structure of human, dog, and cat MHC: HLA, DLA, and FLA. Journal of Heredity 98, 390–399.

Zeisberg, M., Duffield, J.S., 2010. Resolved: EMT produces fibroblasts in the kidney. Journal of American Society of Nephrology 21, 1247–1253.

Please cite this article in press as: Benali, S.L., et al. De novo expression of human leukocyte antigen-DR (HLA-DR) and loss of beta-catenin expression in tubular epithelial cells: A possible event in epithelial–mesenchymal transition in canine renal diseases. The Veterinary Journal (2013), http://dx.doi.org/10.1016/j.tvjl.2013.06.006